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Antidiabetic activity of methanolic bark extract of *Albizia odoratissima* Benth. in alloxan induced diabetic albino mice

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ABSTRACT

Objective: To evaluate the antidiabetic potential of methanolic extract of *Albizia odoratissima* Benth. bark in alloxan induced diabetic mice. **Methods:** Group-Ⅰ (normal control) mice received only basal diet without any treatment. In Group-Ⅱ (Diabetic control) mice, diabetes was induced by alloxan (150 mg/kg *i.p.*) and received only Tween 80, 5% v/v in normal saline. Group-Ⅲ and Group-Ⅳ mice received metformin (10 mg/kg) and gliclazide (10 mg/kg) as standard drugs. Group-Ⅴ and Ⅵ mice received methanolic bark extract of *Albizia odoratissima* at doses of 250 and 500 mg/kg body weight *p.o.*, respectively. **Results:** The results of the study indicates that *Albizia odoratissima* bark extract significantly ($P<0.01$) reduced the blood sugar level. The bark extract also significantly reduced the levels of serum cholesterol, triglycerides, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, alkaline phosphatase and decreases level of total proteins in alloxan induced diabetic mice. **Conclusions:** Methanolic extract of *Albizia odoratissima* has protective effects on the protection of vital tissues (pancreas, kidney, liver, heart and spleen), thereby reducing the causation of diabetes in experimental animals.

1. Introduction

Insulin-dependent diabetes mellitus or type 1 diabetes is an autoimmune disorder caused by destruction of insulin producing β-cells when auto aggressive T-lymphocytes infiltrate the pancreas. This leads to hypoinsulinaemia and thus hyperglycemia[1]. Hyperglycemic condition causes increased glycosylation leading to biochemical and morphological abnormalities due to altered protein structure which over a period of time develops diabetic complications such as nephropathy, retinopathy, neuropathy, and cardiomyopathy[2]. Traditional medicines derived mainly from plants play major role in the management of diabetes mellitus[3,4].

World Health Organization (WHO) has recommended the evaluation of traditional plant treatments for diabetes as they are effective, non-toxic, with less or no side effects and are considered to be excellent candidates for oral therapy[5]. Recently, many reviews on medicinal plants possessing experimental and clinical antidiabetic activity that have been used in traditional systems of medicine[6,7].

Albizia odoratissima Benth. (Mimosaceae) is commonly known as 'Black Siris' and 'Ceylon rose-wood'. The bark is astringent, acrid, cooling, depurative, expectorant and useful in skin diseases, rheumatism, erysipelas cough, bronchitis, diabetes & burning sensation[8]. Thus, the aim of the present study was to investigate the antidiabetic activity of methanolic bark extract of *Albizia odoratissima* in alloxan induced diabetic mice.

2. Materials and methods

2.1. Procurement and Identification of plant material

The bark of plant was collected from the Herbal Nature Park, Yamuna Nagar, during November 2009 and identified as *Albizia odoratissima* (Family: Mimosaceae) by Dr. H.B. Singh, Scientist In-charge, Raw Materials and Museum, National Institute of Science Communication And Information Resources, New Delhi where a voucher specimen (No: NISCAIR/RHM 1382/184) has been deposited for further reference.

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2.2. Preparation of extracts

Bark of *Albizia odoratissima* was dried in shade for two weeks. Dried bark was coarsely powdered, sieved (#40) and stored in an air tight container at room temperature. Dried powder was then extracted sequentially with petroleum ether, chloroform, and methanol using soxhlation method. The extracts were concentrated to dryness using rotary evaporator (Heidolph, model-4011, USA). The yields of various extracts were found to be 1.02% w/w (petroleum ether), 0.82% w/w (chloroform) and 20.34% w/w (methanol). All the extracts were preserved in a refrigerator at 4 ℃. However, only methanolic extract of the bark was selected and evaluated for antidiabetic study.

2.3. Acute toxicity study of the extract

Acute oral toxicity study was performed as per OECD-423 guidelines[9]. Albino mice (n=6) of either sex selected by random sampling technique were used for the study. The animals were kept on fasting for overnight providing only water, after which the extracts were administered orally at the dose level of 5 mg/kg body weight by intragastric tube and observed for 28 days. Mortality was observed at different doses but no mortality was observed at the dose of 500 mg/kg as it was considered as safer dose.

2.4. Oral glucose tolerance test

The effect of methanolic bark extract of *Albizia odoratissima* was evaluated on the glucose (2 g/kg) loaded normal mice. Blood sample was collected from tail vein at the time interval of 0, 15, 30, 45, 60, 75, 90, 105, 120 min. The percentage change in blood glucose levels were monitored at various time intervals after single administration of the extract[10].

2.5. Preparation of glucose solution

The 20% w/v glucose solution was prepared by dissolving 20 g glucose in 100 mL of distilled water.

2.6. Induction of experimental diabetes

Hyperglycemia was induced by injecting alloxan hydrate at a dose of 150 mg/kg intraperitonially[11]. The animals were kept under observation. After 48 h, the animals were tested for glucosuria using Diastex strips. The blood glucose level was checked before and 72 h after alloxon injection to confirm the development of diabetes. The diabetic animals were stabilized for five days and the experiment was started on the next day (day 0). Only those animals which showed blood glucose levels >250 mg/dL were separated and used for the study.

2.7. Experimental design

All the diabetic animals were randomly divided into five groups with six animals each and treated once a day for 28 days as follows:

Group Ⅰ (Normal healthy control): given only vehicle (Tween 80, 1% v/v);

Group Ⅱ served as diabetic control: received only vehicle;

Group Ⅲ diabetic rats: received metformin 10 mg/kg body weight;

GroupⅣ diabetic rats: received gliclazide 10 mg/kg body weight;

Group Ⅴ diabetic rats: received *Albizia odoratissima* bark extract (250 mg/kg body weight);

Group Ⅵ diabetic rats: received *Albizia odoratissima* bark extract (500 mg/kg body weight).

Blood samples were collected on 28th day and centrifuged. Serum was evaluated for the estimation of various biochemical parameters such as glucose, cholesterol, triglycerides, urea, creatinine, total rotein, serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and alkaline phosphatase as per reported methods[12] using ERBA diagnostic kits (Mumbai) using Auto analyzer (ERBA Chem-7). Percentage decreases in the blood glucose levels of extract were monitored at various time intervals after administration of extract at both the two doses and were compared with standard treated groups and control groups.

2.8. Statistical analysis

The Dennett's test was employed for statistical comparison. P<0.05 were considered significant in relation to control and standard. All values are presented as mean±SEM.

3. Results

In normoglycemic method, the immediate effects of methanolic extract significantly decreased the blood glucose levels in normal group mice after 120 minutes. In oral glucose tolerance test (OGTT), the extract showed mild decrease in blood glucose levels in normal animals and showed significant increase in glucose tolerance(P < 0.01), as the blood glucose levels were reduced at higher dose of methanol extract considerably to normal within 120 minutes after drug administration(Table 1).

In alloxan induced antidiabetic mice, bark extract showed significant antidiabetic activity at both the doses *ie*. 250 and 500 mg/kg body weight (P<0.01), which was further evidenced by percentage reduction (49.4%&51.89%) in blood glucose levels on 28th day after administering the extract at both the doses (Table 2). The extract significantlyincreased the body weight of diabetic animal at higher doses (P<0.05 or P <0.01). The extract at lower doses showed significant decrease in weight of liver but not of kidney, pancreas, heart and spleen (P<0.05). However, at higher doses, it showed significant decrease in liver weight (P<0.01). The extract at higher doses showed significant decrease in the weight of kidney and pancreas (P<0.05) with no effect on heart whereas significantly increased the weight of spleen (P<0.01) (Table 3). The methanolic extract at both the doses showed significant increase in serum insulin level (P<0.01). The extract significantly decreased the serum glucose level at both the doses (P<0.05). The extract at both the doses also significantly decreased the serum urea level (P<0.01) (Table 4). It also showed significant decrease in serum creatinine leve(P<0.01 or P<0.05) l but did not show any effect on total protein level at both the doses. The extract at both the doses significantly decreased the SGOT and SGPT levels respectively (P<0.01) (Table 5). The extract did not show any effect on alkaline phosphatase levels at both doses (Table 6).

Table 1

Effect of methanol bark extract of *Albizia odoratissima* on blood sugar level of normal animals and glucose loaded of normal animals (mean±SEM) (*n*=6).

Group	Dose (mg/kg)	Blood sugar level of normal animals (mg/dL)				Glucose loaded normal animals (mg/dL)			
		0 min	30 min	60 min	120 min	0 min	30 min	60 min	120 min
Normal control	5% v/v (Tween 80 in normal saline)	84.50±1.50	81.00±0.90	78.50±2.80	80.16±1.50	78.30±1.60	113.17±3.80	100.67±2.00	90.80±3.50
Methanol extract	250	100.50±2.80	80.30±3.20	90.00±3.30*	79.30±2.80	79.60±1.30	111.00±3.09	92.80±3.00*	85.60±6.09
Methanol extract	500	76.16±1.40	70.30±2.30	78.00±2.60	70.50±1.60**	80.80±0.90	110.20±0.70	93.80±5.60**	82.00±4.28**
Standard– gliclazide	10	83.60±1.40	75.8±1.60	71.22±1.30**	69.30±3.80**	74.50±1.40	116.40±2.98	94.90±0.20**	80.20±2.70**

*P <0.05, ** P <0.01 compared with normal control.

Table 2

Effect of methanol bark extract of *Albizia odoratissima* on the body weight (mean±SEM) (*n*=6).

Group (*n*=6)	Dose (mg/kg)	Body weight (g)				
		0th day	7th day	14th day	21st day	28th day
Normal control	5% v/v (Tween 80 in normal saline)	28.60±1.38	29.20±0.87	28.10±0.76	28.20±1.27	29.00±1.31
Diabetic control	5% v/v (Tween 80 in normal saline)	29.12±2.03	24.95±1.01	26.62±1.45	25.00±3.00	25.48±3.50
Methanol extract	250	28.80±2.08	29.22±1.07	29.09±1.70	30.00±1.20	31.00±2.00
Methanol extract	500	28.70±0.88	29.60±1.79*	31.40±1.44*	30.00±1.11	30.00±2.41**
Standard–metformin	10	29.80±0.83	30.70±0.69**	30.00±0.70**	30.00±1.60	30.20±1.69**
Standard–gliclazide	10	28.70±1.90	29.60±1.50**	32.00±1.04	32.00±2.60	29.20±1.69**

*P <0.05, ** P <0.01 compared with diabetic control.

Table 3

Effect of methanol bark extract of *Albizia odoratissima* on the weight of organs such as liver, kidney, pancreas, heart and spleen (kg)(mean±SEM) (*n*=6).

Group	Dose (mg/kg)	Liver	Kidney	Pancreas	Heart	Spleen
Normal control	5% v/v (Tween 80 in normal saline)	1.17±0.35	0.24±0.02	0.14±0.02	0.22±0.80	0.40±0.02
Diabetic control	5% v/v (Tween 80 in normal saline)	2.06±0.29	0.50±0.10	0.25±0.61	0.40±0.06	0.15±0.02
Methanol extract	250	1.14±0.15*	0.33±0.02*	0.15±0.57	0.38±0.04	0.39±0.01
Methanol extract	500	1.12±0.10*	0.26±0.02**	0.11±0.03**	0.35±0.04	0.33±0.04
Standard–metformin	10	1.19±0.18**	0.28±0.11**	0.17±0.09**	0.38±0.01	0.34±0.01
Standard–gliclazide	10	0.88±0.21**	0.21±0.02**	0.11±0.30**	0.30±0.02	0.39±0.03

*P <0.05, ** P<0.01 compared with diabetic control.

Table 4

Effect of methanol bark extract of *Albizia odoratissima* on the serum cholesterol, triglyceride and glucose, insulin levels (mean±SEM) (*n*=6).

Group	Dose (mg/kg)	Serum cholesterol (mg/dL)	Serum triglyceride (mg/dL)	Serum glucose (mg/dL)	Serum insulin (U/mL)
Normal control	5% v/v (Tween 80 in normal saline)	77.10±1.56	110.36±1.83	105.86±10.91	13.86±1.04
Diabetic control	5% v/v (Tween 80 in normal saline)	194.00±1.82	202.04±9.16	163.28±26.50	2.74±0.18
Methanol extract	250	122.42±17.66**	135.10±5.23	119.70±2.68	9.540±0.43**.
Methanol extract	500	80.19±7.57**	100.08±6.34**	74.15±12.90**	16.14±0.37**
Standard–metformin	10	82.73±6.69**	95.91±8.11**	97.14±9.30**	16.58±1.13**
Standard–gliclazide	10	79.25±3.20**	98.04±1.20**	84.50±9.10 **	16.19±0.53**

*P<0.05, ** P<0.01 compared with diabetic control.

Table 5

Effect of methanol bark extract of *Albizia odoratissima* on the serum urea, creatinine and total protein levels (mean±SEM) (*n*=6).

Group	Dose (mg/kg)	Serum urea (mg/dL)	Serum creatinine (mg/dL)	Serum total protein (g/dL)
Normal control	5% v/v (Tween 80 in normal saline)	45.28±1.40	0.48±0.09	9.31±0.69
Diabetic control	5% v/v (Tween 80 in normal saline)	82.71±7.80	1.57±0.37	4.64±1.53
Methanol extract	250	43.30±2.50**	0.54±0.08**	7.39±0.51*
Methanol extract	500	39.49±1.50**	0.40±0.03**	7.78±0.68*
Standard–metformin	10	39.00±1.60**	0.42±0.15**	7.02±0.71**
Standard–gliclazide	10	43.30±2.60**	0.40±0.02**	7.40±0.75*

*P<0.05, **P<0.01 compared with diabetic control.

Table 6
Effect of methanol bark extract of *Albizia odoratissima* on the SGOT, SGPT and alkaline phosphatase levels (mean±SEM)(n=6).

Group	Dose (mg/kg)	SGOT (IU/L)	SGPT (IU/L)	Alkaline phosphatase (IU/L)
Normal control	5% v/v (Tween 80 in normal saline))	14.60±2.40	13.40±2.50	48.50±7.99
Diabetic control	5% v/v (Tween 80 in normal saline)	48.80±17.40	28.70±9.40	120.60±3.10
Methanol extract	250	27.80±7.57	18.10±1.70	64.80±3.70**
Methanol extract	500	14.18±2.90**	10.70±0.50*	61.20±3.15**
Standard-metformin	10	13.16± 0.83**	11.70±2.26**	60.80±9.60**
Standard-gliclazide	10	15.15±2.40**	15.20±1.20	65.30±3.40**

*P <0.05, ** P <0.01 compared with diabetic control.

4. Discussion

The use of ethnobotanicals has a long folkloric history for the treatment of blood glucose lowering abnormalities[12]. Therefore, the search for more effective and safer antidiabetic/hypoglycaemic agents has continued to be an important area of active research. In the present study, *Albizia odoratissima* (Benth) L. was selected for antidiabetic evaluation owing to its ethno medicinal use in curing diabetes. Therefore, the study was undertaken to justify its claimed use. As a result, methanolic extract of *Albizia odoratissima* bark was prepared by Soxhlet method and stored in refrigerator at 4 ℃. Albino mice were selected as experimental animals for the antidiabetic activity. Acute toxicity study was conducted as per OECD protocols and the methanolic extracts showed no toxicity at the dose of 500 mg/kg as no death was reported for 72 h. The methanolic extract showed significant increase in serum insulin level (P<0.01) whereas significantly decreased the serum glucose level at both the doses (P<0.05). When extract was compared with normal and standard (Metformin and gliclazide), methanolic extract appeared to have maximum antidiabetic activity in normalizing all these parameters. During this prolonged study, various physical parameters were also observed such as body weight, food intake, water intake and weight of internal organs. Generally, body weights are reduced in diabetic patients but in this study, the decrease in body weights were diminished by the extract treatment, thus this effect may be useful for the diabetic patients[13]. Also the changes in food intake, water intake and weight of internal organs were restored to normal by the prolonged effect of extract treatment. Significant reduction was observed in average body weight on administration of methanolic extract at the both the doses(P<0.01). Increase in body weight and decrease in blood glucose might be due to improving the glycemic control mechanisms and insulin secretions from remnant pancreatic-cells in diabetic animals. The results from present study also indicates that *Albizia odoratissima* bark extract may reduce the level of serum cholesterol, triglycerides, SGOT, SGPT, alkaline phosphatase and decrease level of total proteins. It confirms that functions are on the protection of vital tissues (pancreas, kidney, liver, heart and spleen), thereby reducing the causation of diabetes in experimental animals[14].

The results of this investigation indicate that the bark extracts of *Albizia odoratissima* have hypoglycemic effect on alloxan-induced diabetic mice, one possible mechanism of action is due to insulin secretion and improvement of glycogenesis process. The extracts were effective in regulating the biochemical indices associated with diabetes mellitus such as cholesterol, triglycerides, SGOT, SGPT, alkaline phosphatase and also decrease level of total proteins. Further studies are in progress to isolate the active principle(s) from the extracts as well as to elucidate their exact mechanism(s). It is concluded that the plant must be considered as excellent candidate for future studies on diabetes mellitus.

Conflict of interest statement

We declare that we have no conflict of interest.

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